2004

Echinoderm adhesive secretions: From experimental characterization to biotechnological applications

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Abstract

Adhesion is a way of life in echinoderms. Indeed, all the species belonging to this phylum use adhesive secretions extensively for various vital functions. According to the species or to the developmental stage considered, different adhesive systems may be recognized. (1) The tube feet or podia are organs involved in attachment to the substratum, locomotion, feeding or burrowing. Their temporary adhesion relies on a duo-gland adhesive system resorting to both adhesive and deadhesive secretions. (2) The larval adhesive organs allow temporary attachment of larvae during settlement and strong fixation during metamorphosis. (3) The Cuvierian tubules are sticky defence organs occurring in some holothuroid species. Their efficacy is based on the instantaneous release of a quick-setting adhesive. All these systems rely on different types of adhesion and therefore differ in the way they operate, in their structure and in the composition of their adhesive. In addition to fundamental interests in echinoderm bioadhesives, a substantial impetus behind understanding these adhesives are the potential technological applications that can be derived from their knowledge. These applications cover two broad fields of applied research: design of water-resistant adhesives and development of new antifouling strategies. In this context, echinoderm adhesives could offer novel features or performance characteristics for biotechnological applications. For example, the rapidly attaching adhesive of Cuvierian tubules, the releasable adhesive of tube feet or the powerful adhesive of asteroid larvae could each be useful to address particular bioadhesion problems.

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1 Introduction

Among mechanisms allowing marine organisms to attach to or manipulate a substratum, one can distinguish mechanical attachments (*e.g.*, hooks or suckers) from chemical attachments (with adhesive substances) (Nachtigall 1974). The way the former operates is generally obvious whereas the functioning of the latter remains enigmatic. Yet, adhesion (attachment with adhesive substances) is a way of life in the sea. Indeed, representatives of bacteria, protoctists (including macroalgae), and all animal phyla living in the sea attach to natural or artificial surfaces. Adhesion is particularly developed and diversified in invertebrates, who use it during their larval life as well as during their adult life (Walker 1987). It is involved in various functions such as attachment to the substratum, handling of food or building tubes or burrows (Walker 1987, Tyler 1988, Flammang 1996, Whittington and Cribb 2001).

Seawater being a dense medium, denies gravity the power to hold organisms to the bottom; thus, if they want to withstand the hydrodynamism, marine organisms must have adhesive mechanisms. Attachment to the substratum is therefore the most important use of adhesion by marine invertebrates. Adhesion to the substratum may be permanent, transitory or temporary (Tyler 1988, Flammang 1996, Whittington and Cribb 2001). Permanent adhesion involves the secretion of a cement and is characteristic of sessile organisms staying at the same place throughout their adult life (such organisms have representatives among sponges, hydrozoan cnidarians, cirripede crustaceans, bivalve mollusks, tubicolous polychaetes, bryozoans or tunicates) (Walker 1987). Transitory adhesion allows simultaneous adhesion and locomotion: the animals attach by a viscous film they lay down between their body and the substratum, and creep on this film which they leave behind as they move. This type of adhesion is characteristic of invertebrates- mostly small soft-bodied invertebrates such as turbellarians, nemertines, gastrotrichs or polychaetes- moving along the substratum by ciliary gliding (Tyler 1988, Whittington and Cribb 2001). Larger animals such as sea anemones and gastropod mollusks also use transitory adhesion; they move by mean of waves of muscular contractions running along their foot (Walker 1987). Temporary adhesion allows organisms to attach firmly

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but momentarily to a substratum. This type of adhesion is very frequently found in small invertebrates inhabiting the interstitial environment, e.g. in turbellarians, gastrotrichs, nematodes, and polychaetes (Tyler 1988). A few macro-invertebrates -such as some cnidarians and most echinoderms- can also attach and detach repeatedly (Flammang 1996).

Recently, Whittington and Cribb (2001) introduced the term "tissue adhesion" to describe the attachment of symbiotic organisms to the living tissues of their hosts. Although they suggested that tissue adhesion is a fourth type of adhesion, we rather propose that this is a sub-category of either permanent, transitory or temporary adhesion, that should be opposed to adhesion to abiotic substrata. Indeed, examples of tissue adhesion include permanent attachment of parasitic barnacles on whale skin (Ridgway et al. 1997), transitory attachment of parasitic gastropods on echinoderm epidermis (Vaitilingon et al. 2004), or temporary attachment of parasitic monogeneans on the gills or skin of fishes (Whittington and Cribb 2001). There are, however, invertebrate adhesive systems that do not fit into the three types of adhesion described above. These adhesive systems rely on single-use organs or cells and are used in functions other than attachment to the substratum requiring a very fast formation of adhesive bonds. Prey capture by collocyte-bearing tentacles of ctenophorans (Franc 1978, Eeckhaut et al. 1997) is a typical example of this type of adhesion for which we propose the term "instantaneous adhesion".

The phylum Echinodermata is quite exceptional in the sense that most species belonging to this group use adhesion extensively. Moreover, according to the species or to the developmental stage considered, different adhesive systems may be recognized. These include: (1) tube feet or podia, organs involved in attachment to the substratum, locomotion, feeding or burrowing; (2) larval adhesive organs allowing attachment of larvae during settlement and metamorphosis; and (3) Cuvierian tubules, sticky defence organs occurring in some holothuroid species. All these systems rely on different types of adhesion and therefore differ in the way they operate, in their structure and in the composition of their adhesive.

2 Tube feet

Being exclusively benthic animals, echinoderms have activities and adaptations, which are correlated with a benthic existence. Most of these activities, such as attachment to the substratum, locomotion, handling of food and burrow-building, rely on adhesive secretions allowing the animal to stick to or to manipulate a substratum. In post-metamorphic echinoderms, these adhesive secretions are always produced by specialized organs, the podia or tube feet. These are the external appendages of the ambulacral system and are also probably the most advanced hydraulic organs in the animal kingdom. Tube foot attachment is typically temporary adhesion. Indeed, although tube feet can adhere very strongly to the substratum, they are also able to detach easily and voluntarily from the substratum before reinitiating another attachment-detachment cycle (Thomas and Hermans 1985, Flammang 1996)

Tube feet have diversified into a wide variety of morphotypes, which were classified by Flammang (1996) into disc-ending, penicillate, knob-ending, lamellate, ramified, and digitate. In terms of adhesion, however, for practical considerations only disc-ending tube feet involved in attachment to the substratum and locomotion have been studied in details. These tube feet consist of a basal extensible cylinder, the stem, and an enlarged and flattened apical extremity, the disc (Fig. 1A). Tube foot adhesive strength was evaluated by measuring their tenacity, which is the adhesion force per unit area and is expressed in Pascals (Pa). Tenacity of single tube foot has been quantified in several species of asteroids and echinoids. The mean normal tenacities measured on a glass substratum were of 170 kPa in *Asterias vulgaris* (Paine 1926), of 198 kPa in *A. rubens* (Flammang and Walker 1997), and of 59, 120 and 290 kPa in *Arbacia lixula, Sphaerechinus granularis* and *Paracentrotus lividus*, respectively (Santos 2003). Tenacities of whole individuals were also measured in the same echinoid species and respectively average 190, 260 and 330 kPa (Santos 2003). All these values are in the same range as those observed in other marine invertebrates known to adhere very strongly to the substratum (*e.g.*, 230 kPa in limpets, 520 kPa in barnacles, 750 kPa in mussels; see Walker 1987 for review). Tube foot adhesive secretions therefore appear to be well-tailored to provide an efficient attachment to the substratum, allowing echinoderms to resist hydrodynamically generated forces.

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Figure 1

The histological structure of the tube feet is remarkably constant for all echinoderm species. Their tissue stratification consists of four layers: an inner myomesothelium surrounding the water-vascular lumen, a connective tissue layer, a nerve plexus and an outer epidermis covered externally by a cuticle (Flammang 1996). At the level of the tube foot tip, these tissue layers are specialized in adhesion and sensory perception: the connective tissue layer and the nerve plexus are thickened, and the epidermis is differentiated into a well-developed sensory-secretory epithelium. The latter comprises two types of secretory cells: non-ciliated secretory cells (NCS cells) enclosing large heterogeneous granules and ciliated secretory cells (CS cells) enclosing small homogeneous electron-dense granules (see Flammang 1996 for review). In some species, two types of NCS cells co-occur in the sensory-secretory epidermis. The study of the ultrastructure of these different types of secretory cells during a complete cycle of attachment-detachment of the tube foot in *A. rubens* (Fig. 2) demonstrated that they function as a duo-gland adhesive system as originally proposed by Hermans (1983), and in which NCS cells release an adhesive secretion and CS cells a de-adhesive secretion (Flammang et al. 1994, Flammang 1996). The adhesive is present as a thin film between the tube foot cuticle and the substratum and, when detachment occurs, it takes place at the level of the outermost layer of the cuticle, the fuzzy coat, leaving the adhesive material strongly attached to the substratum as a footprint (Fig. 1B,C) (Flammang 1996). In *A. rubens*, polyclonal antibodies have been raised against footprint material and were used to locate the origin of footprint constituents in the tube feet (Flammang et al. 1998a). Extensive immunoreactivity was detected in the secretory granules of both NCS1 and NCS2 cells, suggesting that their secretions make up together the bulk of the adhesive material. No immunoreactivity was detected in the secretory granules of CS cells and the only other structure strongly labelled was the fuzzy coat. This pattern of immunoreactivity suggests that secretions of CS cells are not incorporated into the footprints, but instead might function enzymatically to jettison the fuzzy coat thereby allowing the tube foot to detach (Flammang 1996, Flammang et al. 1998a).

Figure 2

Footprints in echinoderms consist of a sponge-like material deposited as a thin layer on the substratum (Thomas and Hermans 1985, Flammang 1996, Flammang et al. 1998a). Although their diameter is easily measured after staining of the adhesive material (Flammang 1996), footprint thickness is difficult to estimate. Using an interference-optical profilometer, which generated three-dimensional images of the footprint surface, the mean maximum footprint thickness was found to be of 100 nm in the echinoid *P. lividus* and of 230 nm in the asteroid *A. rubens* (Figs. 1 B, C; Santos, Gorb and Flammang, unpublished data). The chemical composition of the footprint material was analysed in *A. rubens*. Leaving inorganic residue apart, this material is made up mainly of proteins and carbohydrates (Flammang et al. 1998a). The protein moiety contains significant amounts of both charged (especially acidic) and uncharged polar residues as well as of cysteine. The carbohydrate moiety is also acidic, comprising both uronic acids and sulphate groups. Adhesive interactions with the substratum could be through ionic bonds presumably involving the acidic residues of both carbohydrate and protein moieties (Waite 1987), whereas cohesive strength could be achieved by intermolecular disulphide bonds. So far, *A. rubens* is the only species in which the tube foot adhesive has been studied biochemically and nothing is known on other echinoderm species. Regarding the asteroids, however, a comparative immunohistochemical study of the tube feet from fourteen species representing five orders and ten families revealed that the adhesives of all these species are closely related, and this independently of the taxon considered, of the species habitat, and of the tube foot morphotype or function (Santos et al. 2004).

Comparison of the composition of the temporary adhesive of *A. rubens* with the one of other marine invertebrates shows that it is closer to the transitory adhesive of limpets, also composed of an association of proteins and acidic glycans, than to the permanent adhesives of mussels and barnacles, made almost exclusively of proteins (Flammang et al. 1998a, Flammang 2003). A similar relationship between non-permanent adhesives is also observed when one compares the amino acid compositions of the temporary adhesive from asteroids to the temporary adhesive from monogenean flatworms, and to the transitory adhesive from limpets (see Flammang 2003). This relationship indicates convergence in composition because of common function (i.e., non-permanent attachment to the substratum) and selective pressures.

3 Larval adhesive organs

For most echinoderms, metamorphosis transform a bilaterally symmetrical and pelagic larva into a radially symmetrical and benthic postmetamorphic individual. Settlement always takes place during the so-called perimetamorphic period (Gosselin and Jangoux 1998, Haesaerts et al. 2003), but either before or after the metamorphic stage according to the class considered (Strathmann 1978). In both cases, adhesive organs attach either the competent larva or the postlarva to the substratum during settlement. In three of the five extant echinoderm classes, these organs are the tube feet, viz. the five primary tube feet of competent echinoplutei in echinoids, the five primary tentacles (and, for some species, two posterior tube feet) of pentactulae in holothuroids, the five primary tube feet and the five first pairs of tube feet of ophiuroid postlarvae (Strathmann 1978). These tube feet are similar in structure and function to tube feet of adults (Cameron and Fankboner 1984, Flammang et al. 1998b). Larval adhesive organs of crinoids and asteroids are, on the other hand, unique and have no equivalent in the postmetamorphic stage (Strathmann 1978).

The perimetamorphic period of crinoids comprises three stages: the doliolaria (free swimming larval stage), the cystidean (attached metamorphic stage) and the pentacrinoid stages (attached postlarval stage) (Mladenov and Chia 1983, Lahaye and Jangoux 1987, Nakano et al. 2003). Competent doliolariae are small barrel-shaped larvae. They possess an attachment complex at their anterior end which consists of a ciliary cap surrounding an apical tuft of elongated cilia and a ventrally located and slightly depressed adhesive pit (Fig. 3A,B). The ultrastructure of this attachment complex has been studied in comatulids (Chia et al. 1986, Jangoux and Lahaye 1990). It is strictly epidermal and made up of elongated ciliated cells associated with a thick basiepidermal nerve plexus. The four cell types forming the complex are sensory cells, covering cells and two types of secretory cells. Sensory cells and secretory cells of the first type occur exclusively in the ciliary cap. The former bear a long vibratile cilium whereas the latter are filled with secretory granules, which contain a flocculent

mucopolysaccharidic material. Secretory cells of the second type are restricted to the adhesive pit where they are the most abundant cell type. These cells are filled with secretory granules with an electron dense fibrillar proteinaceous content. At the beginning of the settlement phase, the doliolaria becomes demersal and brushes the substratum with its apical tuft (sensory structure) (Mladenov and Chia 1983, Lahaye and Jangoux 1988). This implies the occurrence of a mechanism allowing the larva to combine loose adhesion to the substratum with movement. This transitory adhesion is achieved by the combined action of the secretory cells of the ciliary cap that produce a thin mucous film retaining the larva at the water-substratum interface, and of the covering cells whose cilia beat in this mucus (Jangoux and Lahaye 1990, Flammang 1996). When reaching a suitable site, the larva stops moving and turns itself round to have its body directed obliquely (the adhesive pit facing the substratum). It then becomes permanently fixed and transforms into a cystidean larva (Mladenov and Chia 1983, Lahaye and Jangoux 1988). Permanent adhesion starts with the release of the proteinaceous cement by the secretory cells of the adhesive pit and continues during both cystidean and pentacrinoid stages (Chia et al. 1986, Jangoux and Lahaye 1990). After development of the cirri during this last stage, the juvenile detaches from its cemented stalk (Lahaye and Jangoux 1987).

Figure 3

Competent larvae in asteroids are called brachiolariae because they possess a specialized attachment complex on their anterior part comprising three brachiolar arms and an adhesive disc (Fig. 3C) (Barker 1978, Haesaerts et al. 2003). Brachiolar arms are hollow tubular structures occupied by an extension of the larval anterior coelom. Their histological organisation comprises four tissue layers: an inner myomesothelium, a connective tissue layer, a subepidermal nerve plexus and an outer epidermis. Each brachiolar arm is tipped by several sensory-secretory areas named papillae, where both the epidermis and the nerve plexus are greatly thickened. The papillary epidermis encompasses three types of secretory cells (cell type A, B and C), sensory cells, and support cells (Barker 1978, Haesaerts 2000). Type A and B secretory cells are numerous and occupy most of the volume of the papilla while type C secretory cells are scarce and occur only at the base of the papilla. Type A secretory cells bear an apical cilium and contain large ovoid granules that enclose an electron-dense heterogeneous material

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staining histochemically as neutral mucopolysaccharides. Type B secretory cells bear a sub-cuticular cilium and are filled with small granules containing an homogeneous electron-dense material. The adhesive disc is a round, concave structure lying between the brachiolar arms. It is an epidermal structure composed of two main cell types: ciliated secretory cells and support cells (Barker 1978, Haesaerts 2000). The former are full of large secretory granules enclosing a fibrous proteinaceous content of woven aspect. When exploring the substratum, the competent larva orients itself ventral side down and successively attaches and detaches its brachiolar arms (Strathmann 1978, Barker 1978, Haesaerts et al. 2003). Papillae, when making contact with the substratum, are responsible for sensory testing and temporary adhesion. Like adult tube feet, they function as a duo-glandular system with type A and B secretory cells acting as adhesive and de-adhesive cells, respectively (Hermans 1983, Flammang 1996, Haesaerts 2000). In addition, the contents of type A secretory cells cross-react with antibodies raised against tube foot adhesive of *A. rubens*, indicating that adhesives from both brachiolar arms and podia are related to each other and probably share identical molecules, or, at least, identical epitopes on their constituents (Haesaerts 2000). Once the larva has found a suitable site for metamorphosis, brachiolar arms are gradually splayed out, enabling the disc to release its cement (Barker 1978, Haesaerts et al. 2003). This attaches the larva permanently to the substratum and marks the onset of the metamorphic stage. During this stage, tube feet become functional and ultimately help the newly formed postlarva to detach from the cemented disc (Haesaerts et al. 2003).

Among marine invertebrates, crinoids and asteroids are unique in using non-permanent adhesion during settlement (transitory adhesion for the doliolariae and temporary adhesion for the brachiolariae), permanent adhesion for fixation during metamorphosis, and then reversing to non-permanent adhesion for their whole postmetamorphic life (mechanical attachment for the comatulids and temporary adhesion for the asteroids). Indeed, in general, invertebrates which remain mobile as adults use a single type of adhesion throughout their perimetamorphic period. For example, during settlement, pediveligers of gastropods molluscs adhere to the substratum through a viscous film of mucus produced by their foot on which they creep (transitory adhesion; Koehl and Hadfield 2004). This type of adhesion is then conserved up to the adult form

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(Walker 1987). Sessile invertebrates, on the other hand, cannot rely on a single type of adhesion during their perimetamorphic period. These organisms, which as adults use permanent adhesion and live cemented to the substratum, need a non permanent type of adhesion during settlement to enable them to move around while exploring the substratum (Crisp 1984). For premetamorphic attachment, they can therefore use either transitory adhesion like bryozoan larvae or temporary adhesion like barnacle cyprids. From metamorphic attachment onwards, all these organisms then rely on permanent adhesion to remain cemented to the substratum (Crisp 1984).

Adhesion strength of marine invertebrate larvae is difficult to measure due to the small size of these organisms. It can be estimated, however, from the water current required to wash larvae off a glass substratum. Using this technique, the nominal wall shear stress needed to dislodge temporarily attached individuals of the asteroid *Asterina gibbosa* was about 1 Pa for brachiolariae attached by the arms and 6 Pa for postmetamorphic individuals attached by tube feet (Haesaerts, Callow and Flammang, unpubl. data). These values are comparable to those required to dislodge newly settled barnacle cyprids (0.2-8.7 Pa) and nudibranch larvae (4.26 Pa) (Koehl and Hadfield 2004). On the other hand, a nominal wall shear stress of about 40 Pa was needed to detach metamorphic larvae of *A. gibbosa* permanently attached by the disc (Haesaerts, Callow and Flammang, unpubl. data), showing the very high adhesive strength of this permanent adhesive.

4 Cuvierian tubules

Cuvierian tubules are peculiar organs found in several species of holothuroids (sea cucumbers), all belonging exclusively to the family Holothuriidae. Tubules (Fig. 4A) occurring in holothuroids of the genera *Bohadschia*, *Holothuria* and *Pearsonothuria* are expelled as sticky white threads that function as a defence mechanism against predators (Hamel and Mercier 2000, Flammang et al. 2002). Cuvierian tubule adhesion is a typical example of instantaneous adhesion, adhesion being achieved in a matter of seconds (less than 10s; Zahn et al. 1973).

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Figure 4

Cuvierian tubules occur in great numbers (between 200 and 600 in *H. forskali*; VandenSpiegel and Jangoux 1987) in the posterior part of the body cavity of the holothuroid. Proximally they are attached to the basal part of the left respiratory tree and their distal, blind ends float freely in the coelomic fluid. When disturbed, the sea cucumber directs its aboral end toward the stimulating source and undergoes a general body contraction. The anus opens, the wall of the cloaca tears, and the free ends of a few tubules (usually 10 to 20 in *H. forskali*; VandenSpiegel and Jangoux 1987), together with coelomic fluid, are expelled through the tear and the anus. As water from the respiratory tree is forcefully injected into their lumen, the emitted tubules elongate up to 20 times their original length (VandenSpiegel and Jangoux 1987). Upon contact with any surface, the elongated tubules become instantly sticky. The adhesiveness of Cuvierian tubules combined with their tensile strength make them very efficient for entangling and immobilizing most potential predators (VandenSpiegel and Jangoux 1987, Hamel and Mercier 2000). Finally, the expelled tubules autotomize at their attachment point on the left respiratory tree and are left behind as the holothuroid crawls away (VandenSpiegel and Jangoux 1987). After expulsion and autotomy, Cuvierian tubules are readily regenerated. Smooth Cuvierian tubules thus constitute an efficient defensive mechanism. Their large number, sparing use and regeneration dynamics make them a formidable line of defense (Hamel and Mercier 2000, VandenSpiegel et al. 2000).

Cuvierian tubule adhesive strength on glass has been measured in seven species of sea cucumbers belonging to the genera *Bohadschia*, *Holothuria* and *Pearsonothuria* (Flammang et al. 2002). The mean normal tenacities observed varied from about 30 to 135 kPa. These tenacities fall within the range of adhesive strengths described for marine organisms. They lie, however, among the lowest observed values (Flammang 2003).

Cuvierian tubules consist of, from the inside to the outside, an epithelium surrounding the narrow lumen, a thick connective tissue layer and a mesothelium lining the surface of the tubule that is exposed to the coelomic cavity (Fig. 4). The mesothelium is responsible for adhesion. In quiescent tubules, it is a pseudostratified epithelium made up of two superposed cell layers, an outer layer of peritoneocytes and an inner layer of granular cells which is highly folded along

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the long axis of the tubule (Fig. 4B). Granular cells are filled with densely packed membrane-bound granules enclosing a proteinaceous material (Endean 1957, VandenSpiegel and Jangoux 1987). During elongation, the structure of the mesothelium is modified: the protective outer layer of peritoneocytes disintegrates and the granular cell layer, now unfolded, thus becomes outermost on the tubule. Granular cells empty the contents of their granules when the elongated tubule comes into contact with a surface, resulting in adhesion (VandenSpiegel and Jangoux 1987, De Moor et al. 2003).

Table 1

In *H. forskali*, tubule print material -i.e., the adhesive left on the substratum after mechanical detachment of the tubule- is composed of 60% protein and 40% neutral carbohydrate, a composition which is unique among the adhesive secretions of marine invertebrates (De Moor et al. 2003). The proteinic nature of the adhesive material is confirmed by the observation that proteolytic enzymes reduce the adhesive strength of Cuvierian tubules in *H. forskali* (Zahn et al. 1973). The amino acid compositions of the protein fraction in *H. forskali*, *H. leucospilota*, *B. subrubra* and *P. graeffei* indicates that their adhesives are closely related. All are rich in small side-chain amino acids, especially glycine, and in charged and polar amino acids (Table 1). Their compositions differ, however, from those of every other marine bioadhesive (Flammang 2003). Only a small fraction of the Cuvierian tubule adhesive can be extracted using denaturing buffers. This soluble fraction contains several proteins with different molecular masses but with closely related amino acid compositions, resembling to the one of the whole adhesive (De Moor et al. 2003). As for the tube foot adhesive, charged and polar amino acids are probably involved in adhesive interactions with the substratum through hydrogen and ionic bonding (Waite 1987). Small side-chain amino acids, on the other hand, are often found in large quantities in elastomeric proteins (Tatham and Shewry 2000). These proteins are able to withstand significant deformations without rupture before returning to their original state when stress is removed (Smith et al. 1999). The composition of Cuvierian tubule adhesive has therefore all characteristics of a strong and resistant underwater adhesive.

5 Applications of marine bioadhesives

In addition to fundamental interests in marine bioadhesives, a substantial impetus behind understanding these adhesives are the potential technological applications that can be derived from their knowledge. These applications cover two broad fields of applied research: design of water-resistant adhesives and development of new antifouling strategies.

Sessile organisms such as mussels, barnacles or tube-dwelling worms are important macro-foulers and their adhesives are secreted as a fluid and then gradually solidify to form a cement possessing high adhesive and cohesive strengths (Walker 1987). Most studies of invertebrate adhesive systems have therefore focused on the characterization of the permanent adhesives from these organisms (see, e.g., Taylor and Waite 1997, Kamino et al. 2000). The best-characterized permanent adhesive is the one from the blue mussel, *Mytilus edulis*. In this species, several proteins have been identified and characterized that co-occur as a complex blend in the byssal adhesion plaques (Waite 2002). So far, however, only one of these proteins (*Mytilus edulis* foot protein 1; Mefp-1) has been used in biotechnological applications: in the form of crude or recombinant preparations of the protein, and in the form of chemically-synthesized derived peptides (Burzio et al. 1997, Deming 1999; Taylor and Weir 2000).

5.1 Design of water-resistant adhesives

The fact that marine invertebrates produce adhesives that act in presence of water has aroused increasing scientific and technological attention because water, including moist air, is usually the commonest subverter of man-made adhesive joints (Kinloch 1987, Waite 1987). Biomimetic materials inspired by marine adhesives are therefore sorely needed for applications in watery environments. Such materials could be used in underwater construction of course, but the most important applications are certainly to be found in the biomedical field (Strausberg and Link 1990, Peppas and Langer 1994, Taylor and Waite 1997). Indeed, the environment of the sea is similar in many ways to the internal environment of mammalian organisms. Tissues are bathed in fluids with pH and

ionic composition similar to saltwater. Theoretically, attachment mechanisms that some marine invertebrates have evolved to survive can be useful as biological adhesives for *in vitro* as well as *in vivo* uses.

For *in vitro* techniques, a crude preparation of Mefp-1 has been developed as a cell and tissue adhesive (Cell-Tak™, BD Biosciences) for immobilization of biologically active moieties to inert substrata (Benedict and Picciano 1989, Taylor and Waite 1997). Cell-Tak is used to attach cells or tissue sections to many types of surfaces, including plastic, glass, metal, and biological materials. It can simplify the manipulation of biological samples for a number of techniques, including *in situ* hybridization, immunoassays, microinjection, immunohistochemistry, and establishing primary cells in culture (Benedict and Picciano 1989, Burzio et al. 1997, Taylor and Waite 1997). Cell-Tak has been used successfully with a variety of healthy cell types, tumor cells, permeabilized cells, and subcellular components (Taylor and Waite 1997). This broad range of applications is explained by the fact that Mefp-1, in contrast to cell adhesion molecules, acts as a non specific attachment factor that fastens onto a variety of functional groups present and accessible on the surface of all cells and tissues (Taylor and Waite 1997).

Surgical or topical reconnection of severed tissues is essential for restoration of their structure and function. The most widely used methods for joining tissues focus on mechanical fasteners such as sutures and staples. Surgical adhesives, however, provide attractive alternatives to mechanical fastening (Strausberg and Link 1990, Albala 2003, Ninan et al. 2003, Singer and Thode 2004). In addition to their rapid application, they are particularly useful in tissues which are difficult to reach, too difficult to cauterize or too delicate to withstand suturing. Moreover, they are relatively painless to apply and may not require the use of painful local anesthetics. They are also biodegradable, eliminating the need for suture removal. Currently, two types of surgical adhesives are commercially available, fibrin-based adhesives (see, *e.g.*, Albala 2003) and cyanoacrylate-based adhesives (see, *e.g.*, Singer and Thode 2004), which have been used successfully in a growing number of surgical procedures. There are still several applications, however, for which these adhesives cannot be used, e.g. in areas continuously bathed in body fluids (mucous membranes, bladder, etc) (Albala 2003, Ninan et al. 2003, Singer and Thode 2004). Marine bioadhesives could be ideal candidates

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for such applications because they function in aqueous environment, they possess the appropriate adhesive and cohesive properties, and they are ultimately biodegradable (Strausberg and Link 1990, Ninan et al. 2003). However, any adhesive material targeted for medical applications should also be biocompatible (non-toxic, low immunogenicity) and should not interfere with the natural healing process (Strausberg and Link 1990). Mefp-1 seems to be biocompatible and non-toxic *in vitro* (Benedict and Picciano 1989). However, much has still to be learned before clinical trials are performed for human applications (Burzio et al. 1997). Dentistry is another field in which there is current demand for nontoxic bioadhesives able to form durable adhesive bonds in the aqueous environment of the mouth (Peppas and Langer 1994, Burzio et al. 1997).

5.2 Development of new antifouling strategies

Biofouling is one of the most important problems currently facing marine technology. In the marine environment any solid surface will become fouled. Materials submerged in seawater experience a series of discrete physical, chemical and biological events, which results in the formation of a complex layer of attached organisms known as biofouling (Abarzua and Jakubowski 1995, Callow and Callow 2002). Until now, antifouling methods involved the use of toxic self-polishing paints releasing tributyltin (TBT) or cuprous oxide. However, the impact of these compounds on the environment has led to a ban on TBT-containing paints utilization and to a close monitoring of copper discharge from antifouling paints (Callow and Callow 2002). Fouling control is therefore increasingly a problem of managing adhesion, and molecular understanding of how marine bioadhesives work should open up novel technologies intended to specifically intervene in organism attachment (Taylor and Waite 1997, Callow and Callow 2002). One of these technologies is the use of enzyme-leaching paints. Indeed, it has been shown that enzymes can be active in a paint coating and hydrolyze the attachment glue which is secreted by the fouling organisms (Abarzua and Jakubowski 1995). Moreover, enzymes are completely degraded in seawater within several days and they are non-toxic for marine life. Another technology is based on the development of fouling-release coatings such as silicone coatings (Callow and Callow 2002). These coatings, which exhibit low

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critical surface tensions, function by minimizing the adhesion strength of attached organisms. Fouling organisms can settle on the coating but are then removed by either mechanical or hydrodynamic cleaning. Paradoxically, a recently-developed fouling-release coating was based on a marine bioadhesive possessing strong fouling characteristics. Dalsin et al. (2003) synthesized hybrid molecules by combining a decapeptide derived from Mefp-1 and poly(ethylene glycol), and used these molecules to modify surfaces. The strategy exploits the adhesive characteristics of the decapeptide to anchor poly(ethylene glycol) onto surfaces, rendering the surfaces resistant to cell attachment *in vitro*. In the future, it may also be employed for preventing mussels, barnacles and other fouling organisms from attaching to ship hulls, piers, and other man-made structures (Dalsin et al. 2003).

5.3 The potential of echinoderm adhesives

In view of the examples mentioned above, it appears that Mefp-1 has been used succesfully for several applications. However, some limitations have come out such as requirement of post-translational modifications to certain amino acids, or the need for a separate enzyme for curing (Strausberg and Link 1990). New adhesive molecules that could overcome these problems are therefore sought. In this context, echinoderm adhesives could offer novel features or performance characteristics for biotechnological applications. For example, the rapidly attaching adhesive of Cuvierian tubules, the releasable adhesive of tube feet or the powerful adhesive of asteroid larvae could each be useful to address particular bioadhesion problems. If the tube foot de-adhesive secretion proves effectively to be an enzyme, its incorporation into antifouling paints would be particularly relevant as many fouling larvae use temporary adhesion during settlement (Crisp 1984).

Work is currently in progress to identify, purify and characterize the different molecules involved in echinoderm adhesion. The complete elucidation of their structure and physico-chemical characteristics is an obligatory prerequisite before any application can be envisaged.

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6 Acknowledgements

7 References

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Figure legends

Figure 1. Tube foot adhesion and adhesive in the echinoid *Paracentrotus lividus* (originals). SEM photograph of a disc-ending tube foot (**A**), and 3-D and 2-D topographical views of the adhesive footprints deposited on a glass substratum by this tube foot (**B** and **C**, respectively).
D disc, S stem, SSE sensory-secretory epidermis.

Figure 2. Ultrastructure of the tube foot sensory-secretory epidermis in the asteroid *Asterias rubens* during an attachment-detachment cycle (originals). TEM photographs showing the non-ciliated secretory cells (**A**) and the ciliated secretory cells (**B**) before attachment. During attachment to the substratum, non-ciliated secretory cells release some of their granules (**C**) while ciliated secretory cells remain unchanged (not illustrated). After voluntary detachment from the substratum, ciliated secretory cells have released their most apical granules (**D**).
CS ciliated secretory cell, CU cuticle, FC fuzzy coat, NCS1 type 1 non-ciliated secretory cell, NCS2 type 2 non-ciliated secretory cell, P pore, SCC subcuticular cilium, SG secretory granule.

Figure 3. Larval adhesive organs of crinoids and asteroids. SEM photographs of the doliolaria larva of *Antedon bifida* (**A**) and of its anterior adhesive pit (**B**) (from Lahaye, 1987), and of the brachiolaria larva of *Asterias rubens* (**C**) (original).
AD adhesive disc, AdP adhesive pit, AP apical papilla, AT apical tuft, CC ciliary cap, LBA lateral brachiolar arm, LP lateral papilla, M mouth, MBA median brachiolar arm, PL preoral lobe, V vestibule, 1-4 ciliary bands.

Figure 4. Morphology of the Cuvierian tubules of *Holothuria impatiens* (originals). SEM photograph of a transversally-sectioned tubule (**A**), and longitudinal histological section showing the arrangement of the tissue layers (**B**).
CTL connective tissue layer, IE inner epithelium, L lumen, ML muscle layer, M mesothelium.

Table 1. Amino acid compositions of adhesive prints from the Cuvierian tubules of several species of holothuroids (values in residues per thousand).

Amino acid	*Holothuria forskali*[1]	*Holothuria leucospilota*[2]	*Bohadschia subrubra*[2]	*Pearsonothuria graeffei*[2]
HYP	0	24	8	8
ASX	78	74	64	62
THR	87	69	65	80
SER	60	42	58	58
GLX	91	122	106	124
PRO	55	74	69	63
GLY	266	267	298	254
ALA	88	115	91	85
CYS/2	14	3	9	4
VAL	38	29	35	37
MET	10	9	1	9
ILE	28	24	25	32
LEU	37	31	37	38
TYR	20	14	17	17
PHE	20	16	20	20
HIS	26	13	8	20
HLYS	0	5	12	3
LYS	31	12	29	22
ARG	50	57	46	63

[1]De Moor et al. 2003; [2]Flammang and Waite, unpublished data

A

SSE

100μm

B

+0.05469

μm

-0.02565
1.06

mm

0.00

0.00   mm   1.41

C

+0.05469

Peak

μm

Valley

-0.02565
Width: 0.10 mm

A — FC, CU, NCS1, NCS2, 1μm

B — FC, CU, SG, CS, NCS1, 1μm

C — P, NCS2, SG, NCS1, 1μm

D — P, SG, SCC, NCS2, CS, 0,5μm

A

M

200 µm

B

M

CTL

ML

L

IE

100 µm